Studies on blood coagulation factor V

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III — Kinetics of the estimation of factor V

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It has been shown by Hemker and al. (1968) that a linear relationship exists between the thromboplastin time and the inverse of the concentration of any of the coagulation factors determining the reaction velocity in the extrinsic system, i.e. the factors II, V, VII and X. In this article we will give further experimental results concerning the relation between the concentration of factor V and the coagulation time in the extrinsic system, obtained by using different factor V deficient plasmas as a reagent and different plasmas containing factor V as a substrate.

MATERIALS AND METHODS

All determinations of coagulation times were carried out in the following reaction mixture :

- 0.1 ml factor V deficient plasma **(reagent)** ;
- 0.1 ml human brain thromboplastin (Owren and Aas, 1951) ;
- 0.1 ml preparation containing factor V **(substrate)** ;
- 0.1 ml $CaCl_2$ 25 mM.

The time between recalcification and the first appearance of a fibrin clot was measured at 37 °C with the aid of a Kolle hook.

Factor V-deficient plasma was prepared according to Quick (1943) or according to Borchgrevink (1960), or by Ba-Stearate adsorption (Kahn and Hemker, 1970).

Normal pool plasma was mixed citrated plasma from 30 healthy donors. Coumarin plasma was human citrated plasma from patients under long-term anticoagulant treatment.

The residual amount of factor V in a reagent was estimated as described by Hemker and al. (1965). The theoretical background and proof of this method are to be found in ref. 5 and 6. The practical procedure is as follows. By dilution of a normal pool plasma a series of known concentrations of factor V is prepared. The clotting time (t_c) belonging to each of these concentrations (C) is estimated. Then the clotting time (t_1) is estimated, which is obtained when buffer is added as a substrate in the test, instead of a sample containing factor V. This clotting time obviously is determined by the factor V activity of the reagent (L).

Then for eacht t_c the value of $(t_1 - t_c)/C$ was calculated using the fixed value of t_1 and the values of t_c and C

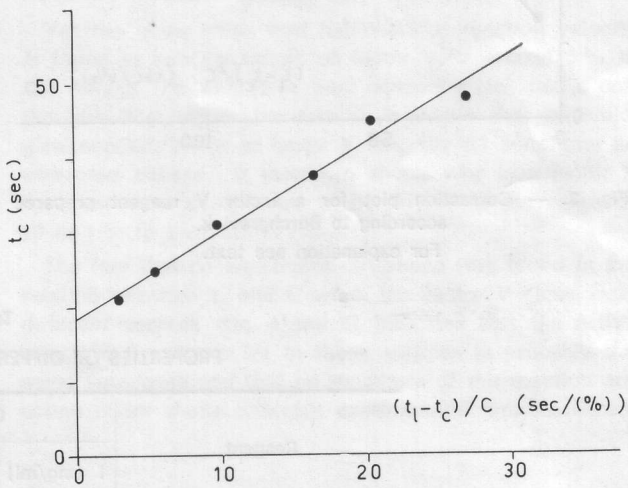

Fig. 1. — Correction plot of a batch of Ba-Stearate adsorbed plasma.

For explanation see text. From the slope of this line the concentration of factor V in the reagent could be calculated to be 1.28 %.

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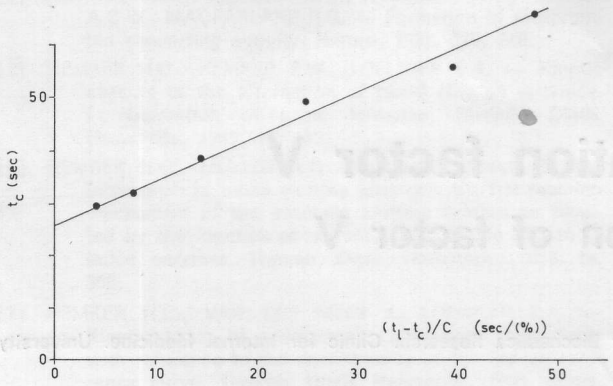

Fig. 2. — Correction plot of a batch of old oxalated plasma. For explanation see text. From the slope of this lite the concentration of factor V in the reagent could be calculated to be 0.86 %.

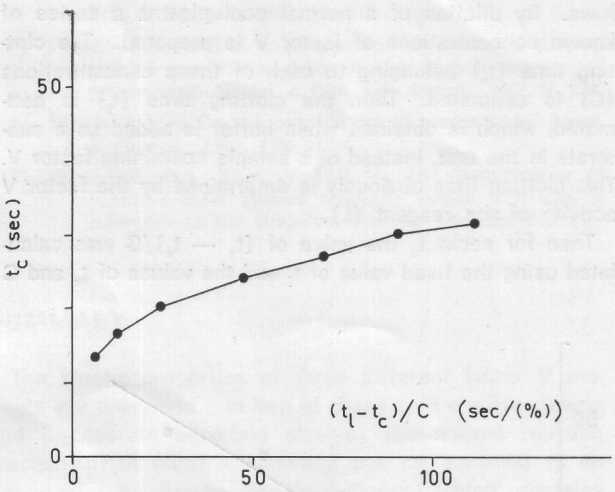

Fig. 3. — Correction plot for a factor V reagent prepared according to Borchgrevink.
For explanation see text.

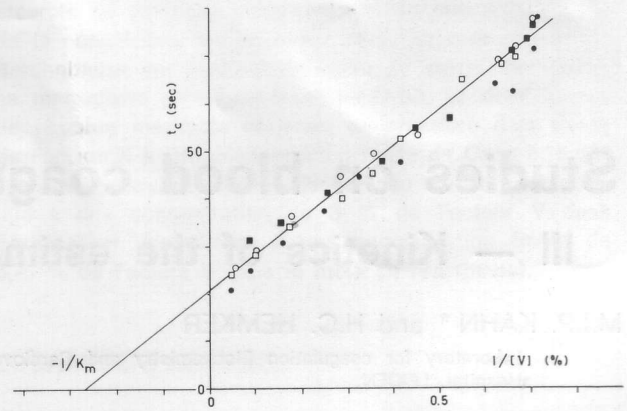

Fig. 4. — The plot of clotting time versus the inverse of concentration of factor V obtained with old oxalated plasma. The points are the means of tenfold estimations carried out with :

- ● normal pool plasma ;
- o normal oxalated plasma ;
- □ $BaSO_4$ adsorbed oxalated plasma ;
- ● coumarin plasma.

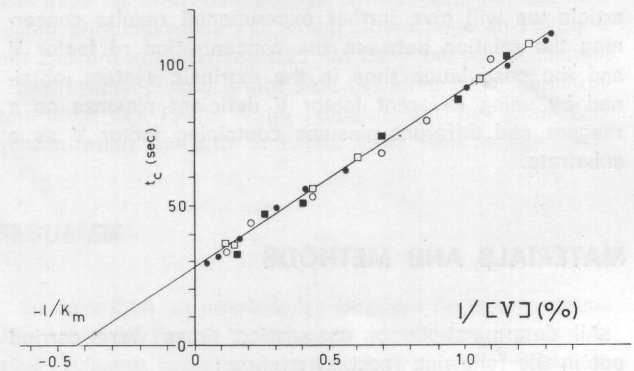

Fig. 5. — The plot of clotting time versus the inverse of concentration of factor V obtained with Ba-Stearate adsorbed plasma. The points are the means of tenfold estimations carried out with :

- ● normal pool plasma ;
- o normal oxalated plasma ;
- □ $BaSO_4$ adsorbed oxalated plasma ;
- ● coumarin plasma.

Table I

PROPERTIES OF DIFFERENT FACTOR V REAGENTS

Reagent	Concentration of coagulation factor				
	I (mg/ml)	II (%)	V* (%)	VII/X (%)	K_m** (%)
Old oxalate	3.7	150	0.86	130	3.7
Borchgrevink	1.6	72	—	120	1 to 4
Ba-Stearate adsorbed	3.2	42	1.12	70	2.3

* Estimated by the graphical method as indicated under materials and methods.

** Concentration of factor V at which half-maximal reaction velocity was observed. Since the factor V activity of Borchgrevink's reagent could not be estimated for this reagent, the value can only be a rough approximation.

belonging together ; t_c is then plotted against $(t_1 - t_c)/C$. This is called the **« correction plot ».** The slope of the resulting line has the dimension of a concentration and can be shown to indicate the magnitude of L, i.e. the concentration of the factor V activity present under conditions under which t_1 was obtained, i.e. the factor V activity in the reagent.

EXPERIMENTAL RESULTS

Three different reagents were compared :

a) a batch of old oxalated plasma ; b) plasma incubated with R.V.V. according to Borchgrevink ; c) Ba-Stearate adsorbed plasma.

The coagulation times obtained with these reagents and normal pool plasma in various dilutions were estimated. To estimated. To estimate the residual amount of factor V activity in the reagent, a correction plot was constructed for each reagent (fig. 1, 3). This plot was a straight line for the old oxalate plasma and the Ba-Stearate adsorbed plasma, but not for Borchgrevink's reagent, which implies that the factor V activity of Borchgrevink's reagent could not be estimated.

Once the concentration of factor V in the reagent being known, it is possible to calculate the true concentration of factor V in a test when a known amount of factor V is added to the reaction mixture in the form of a dilution of normal pool plasma. This true concentration of factor V equals the sum of the amount of factor V added with the sample and the amount present in the reagent.

When the coagulation time obtained with either old oxalate plasma or Ba-Stearate adsorbed plasma was plotted against the inverse of the true concentration of factor V, a straight line resulted (fig. 4 and 5).

K_m, the concentration of factor V at which half-maximal reaction velocity is found, was calculated from these graphs. The properties of the different reagents are summarized in Table I.

DISCUSSION

The calculation of the residual amount of factor V in a factor V reagent is based upon the kinetic evaluation of the normal coagulation mechanism (Hemker and Muller, 1968). The fact that the method does not work in the case of Borchgrevinks reagent indicated that the Russell's viper venom added in this reagent disturbs the normal coagulation mechanism in such a way that its kinetic properties are basically altered. This is to be expected because R.V.V. influences at least two different coagulation factors, viz. factors V and X. Factor X is activated by R.V.V. (Macfarlane, 1964 ; Williams and Esnouf, 1962) and factor V is first activated (Bergsagel, 1965) and then destroyed (Borchgrevink, 1960). It is the latter property of R.V.V. that is employed in preparing Borchgrevink's reagent. When normal plasma is added all three actions of R.V.V. will again start to play a role, resulting in a complicated overall pattern. Borchgrevink's reagent is therefore unsuitable for the evaluation of the kinetics of factor V action.

Both old oxalate plasma and Ba-Stearate adsorbed plasma are suitable for this purpose. Obtaining a good reagent from oxalated plasma by incubating under oxygen at 37 °C was in our hands a hazardous procedure, but when the procedure is performed successfully, an excellent reagent can result. Ba-Stearate adsorption is a reproducible procedure method that gives a good reagent. With both these reagents a Lineweaver Burk plot can be constructed ; and the concentration of factor V at which half-maximal reaction velocity was found (K_m) was about 3 %. The difference in K_m found with the different reagents is probably not significant.

Contrary to the K_m found in simpler enzymatic reactions (Dixon and Webb, 1958), not too much significance should be given to the value of K_m found as an inherent property of the enzyme system under study. The physical meaning of this K_m is that it indicates the equilibrium constant of the reaction in which prothrombinase is formed, i.e.

$$V + X_a + \text{Ph. lip.} + Ca^{++} \leftrightarrows \text{Prothrombinase}$$

(c.f. Hemker and al., 1967).

But in the case under study the system was tested at a fixed concentration of factor X and Ca^{++} and phospholipid. The apparent K_m resulting will thus include these variables. Moreover, only one kind of phospholipid was tested. More series of experiments will have to be done to evaluate the constants of the reaction in more detail.

Yet the observation that half-maximal reaction velocity is found at concentrations of factor V or around 3 % in the sample (i.e. at 0,75 % final concentration) has a certain practical value, because it indicates that physiological concentrations of factor V (i.e. 100 %) constitute an enormous excess. It therefore shows why in a factor V estimation the sample has to be diluted to the range of 10 to 1 % to give useful results.

The fact that no significant difference was found in the relation between t_c and C when the factor V came from different sources (fig. 4 and 5) indicates that the active principle (i.e. factor V) in these sources is probably the same substance and that no modifiers of the reaction are acting under these different experimental circumstances.

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SUMMARY

The kinetic properties of three different factor V reagents are described. In two of these (old oxalate plasma and Ba-Stearate adsorbed plasma) the normal reaction mechanism of blood coagulation can be assumed to be operative. In Borchgrevink's reagent, which contains Russell's viper venom, this reaction mechanism is disturbed. Half-maximal reaction velocity in the normal reaction mechanism, with an excess of factor X present at a final concentration of 6.25 mM Ca^{++} with human brain thromboplastin is found at a 3 % concentration of factor V in the sample, i.e. at a final concentration of 0,75 % factor V in the reaction mixture.

RESUME

Les propriétés cinétiques de 3 différents réactifs du Facteur V sont décrites. On peut admettre qu'avec deux d'entre eux (plasma oxalaté vieilli et plasma adsorbé sur stéarate de baryum) le mécanisme de réaction normale de la coagulation sanguine est actif. Avec le réactif de Borchgrevink qui contient du venin de vipère de Russel, ce mécanisme réactionnel est perturbé. La demi-vitesse de réaction maximale normale, en présence d'un excès de Facteur X à une concentration finale de Ca^{++} 6.25 nM avec de la thromboplastine de cerveau humain, est obtenue à une concentration de 3 % de Facteur V dans l'échantillon, c'est-à-dire à une concentration finale de 0,75 % de Facteur V dans le mélange réactionnel.

ZUSAMMENFASSUNG

Die kinetischen Eigenschaften von 3 verschiedenen Faktor V-Reagenzien werden beschrieben. Bei 2 von diesen (gealtertes Oxalat-Plasma und Ba-Stearat — absorbiertes Plasma) kann der normale Blutgerinnungsmechanismus als wirksam angenommen werden. Bei dem Reagenz nach BORCHGREVINK, das RUSSEL- Schlangengift enthält, ist dieser Wirkungsmechanismus gestört. Die maximale Halbwert-Reaktionsgeschwindigkeit bei der normalen Wirkungsweise mit einem Überschuss an Faktor X mit Endkonzentration von 6,25 nM Ca^{++} bei menschlichem Hirn-Thromboplastin ergibt sich bei einer 3 % igen Konzentration des Faktor V im Präparat, d.h. bei einer Endkonzentration von 0,75 % Faktor V im Reaktionsgemisch.

RESUMEN

Se describen las propiedades cinéticas de tres reactivos diferentes del Factor V. Se puede admitir que con dos de ellos (plasma oxalatado envejecido y plasma adsorbido sobre estearato de bario), el mecanismo de reacción normal de la coagulación sanguínea es activo. Con el reactivo de Borchgrevink, que contiene veneno de vívora de Russell, este mecanismo reaccional se halla perturbado. La media velocidad de reacción máxima normal en presencia de un exceso de Factor X, con concentración final de Ca^{++} 6.25 nM y con tromboplastina de cerebro humano, se obtiene a una concentración de 3 % de Factor V en la muestra, es decir, a una concentración final de 0.75 % de Factor V en la mezcla reaccional.

Резюме Описываются кинетические свойства трех различных реагентов фактора V . В двух из них - старый оксалат плазмы и BA-стеарат адсорбированной плазмы - нормальный механизм коагуляции крови можно считать действенным. В реагенте Борхгревинк,содержащим яд гадюки Рассел,этот механизм реакции нарушен.